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Ontogeny of adipokine expression in neonatal pig adipose tissue ☆

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ABSTRACT

This study examined ontogeny of development for a range of adipokines in neonatal adipose tissue. Pigs (*Sus scrofa*) were selected across six litters for sampling subcutaneous (SQ) and perirenal (PR) adipose tissues at d1, d4, d7 or d21 of age and total RNA extraction. Reverse transcription and real-time PCR were used to quantify mRNA abundance for: leptin, adiponectin, interleukin 1β (IL-1β), IL-6, IL-8, IL-10, IL-15, tumor necrosis factor α (TNFα), haptoglobin, vascular endothelial growth factor (VEGF), macrophage migration inhibitory factor (MIF), monocyte chemoattractant protein 1 (MCP1) and cyclophilin. Leptin, adiponectin and IL-15 expression increased from d1 to d 21 of age in both SQ and PR. Haptoglobin, VEGF, MIF and IL-8 expression decreased between d1 and d4 of age in SQ. TNFα expression was unchanged from d1–7 and then increased at d21. IL-1β, IL-6 and IL-10 expression were unchanged with age in SQ; whereas IL-1β and IL-6 mRNA abundance in the PR increased with age. Analysis of the mRNA abundance for these adipokines within adipose tissue from d1 to d21 of age demonstrated that neonatal development of adipokine expression varies among the different adipokines and the internal and external sites of adipose tissue deposition (PR versus SQ).

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1. Introduction

The neonatal period of adipose tissue development is a time of rapid transition. Pigs are born with less than 1–2% body fat, which rapidly increases to approximately 15% by the end of weaning and this fat becomes a major energy source for neonatal survival (Anderson and Kauffman, 1973; Mersmann et al., 1975). The primary change during the perinatal and neonatal period of growth in adipose tissue is the differentiation and enlargement of adipocytes, with the rapid accumulation of lipids and proteins that functionally identify cells as adipocytes (Anderson and Kauffman, 1973; Hausman and Martin, 1981; Ding et al., 1999). The development of adipose tissue is accompanied by the expression of a variety of paracrine and endocrine secretions. Peptides secreted by adipose tissue are now called "adipokines" and may include growth factors, hormones, and cytokines (Trayhurn and Wood, 2004). *In vitro* experiments have demonstrated that neonatal adipose tissue can express the adipokines insulin-like growth factors I and II, and tumor necrosis α (TNFα); (Gaskins et al., 1990; Tchoukalova et al., 2000; Hausman et al., 2002). An *in vivo* study has shown that leptin is also expressed by neonatal pig adipose tissue (Mostyn et al., 2005). Microarray and proteomic analysis has detected numerous proteins expressed by neonatal adipose tissue, including the adipokines TNFα, interleukin-1β (IL-1β), IL-6, IL-8 and IL-15 among others (Hausman et al., 2006). Studies with pigs older than 6 months of age have demonstrated that porcine adipose can express IL-6 and IL-15, TNFα, adiponectin, and resistin (Chen et al., 2004; Jacobi et al., 2004; Ajuwon and Spurlock, 2005).

Furthermore, studies in other species post-weaning have shown *in vitro* expression of numerous adipokines. Fain et al. (2004b) demonstrated that human adipose tissue expresses IL-1β, IL-8, IL-10, hepatocyte growth factor, vascular endothelial growth factor (VEGF), and plasminogen activator inhibitor 1 (PAI-1). Additional inflammatory cytokines secreted by adipose tissue include monocyte chemoattractant protein-1 (MCP1), macrophage migration inhibitory factor (MIF), haptoglobin, nerve growth factor, transforming growth factor β, and others (Trayhurn and Wood, 2004; Fain, 2006; Guzik et al., 2006; Trayhurn et al., 2006).

These various studies have demonstrated that adipose tissue is an active secretory tissue that releases a variety of adipokines with metabolic and immunological actions throughout the body. Most of these proteins have been detected in the pig, but it is not known if many of them are produced by adipose tissue *in vivo*. Studies are needed to elucidate the full complement of adipokines produced by neonatal adipose tissue and their ontogeny of development. These studies are necessary prior to evaluating the response of adipokines to various types of stress during this period of rapid adipose tissue development. Thus, the present study was designed to characterize

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the development of adipokine expression during neonatal adipose tissue development in swine.

2. Materials and methods

Six sows (*Sus scrofa*) of similar genetics, body weight and parity were fed a standard swine diet during gestation. All sows had been bred by the same boar. Sows were individually housed and were monitored to ensure collection of piglets within the first 24 h of birth (day 1). Piglets were weighed on day 1 (d1) and then randomly assigned across litters for sampling at d1, d4, d7 or d21 of age. Pigs used in this study were born at a mean body mass of 1.7±0.1 kg and reached a mass of 7.2±0.7 kg on d21 at the termination of the study. All sampling was performed between 0900 and 1100 h and animals were only sampled in the fed state. Tissue sampling was performed following euthanasia induced with pentobarbital sodium (200 mg/kg). Animal handling and euthanasia procedures were approved by the USDA–ARS Beltsville Area animal care and use committee. Dorsal subcutaneous (SQ) adipose tissue samples were collected between the second and fourth thoracic vertebrae while perirenal (PR) adipose tissue was collected from around both kidneys. No PR adipose was apparent in the d1 pigs and could not be collected, so all PR sampling was begun at d4. Tissues were diced and frozen in liquid nitrogen immediately upon removal from the carcass and stored at −80 °C. Total RNA was isolated using Qiagen RNeasy spin columns according to the manufacturer's protocol (Qiagen, Valencia, CA, USA). Integrity of RNA was assessed via agarose gel electrophoresis and RNA concentration was determined spectrophotometrically using A260 and A280 measurements.

Table 1
Primer sequences for PCR of porcine adipokines

Gene	Primer	Primer Sequences	Amplicon Size	GenBank Accession No.
Leptin	Sense	5′-TGACACCAAAACCCTCATCA-3′	348	U59894
	Antisense	5′-GCCACCACCTCTGTGGAGTA-3′		
Adiponectin	Sense	5′-TACCCCAGGCCGTGATGGCA-3′	213	AY135647
	Antisense	5′-ACCCGAGTCTCCAGGCCCAC-3′		
Tumor Necrosis Factor α	Sense	5′-CCCCTCTGAAAAAGACACCA-3′	180	X54001
	Antisense	5′-TCGAAGTGCAGTAGGCAGAA-3′		
Interleukin1β	Sense	5′-CAGCCATGGCCATAGTACCT-3′	216	M86725
	Antisense	5′-CCACGATGACAGACACCATC-3′		
Interleukin 6	Sense	5′-ATGGCAGAAAAAGACGGATG-3′	215	NM214399
	Antisense	5′-GTGGTGGCTTTGTCTGGATT-3′		
Interleukin 8	Sense	5′-GGCAGTTTTCCTGCTTTCT-3′	154	X61151
	Antisense	5′-CAGTGGGGTCCACTCTCAAT-3′		
Interleukin 10	Sense	5′-AGCCAGCATTAAGTCTGAGAA-3′	394	L20001
	Antisense	5′-CCTCTCTTGGAGCTTGCTAA-3′		
Interleukin 15	Sense	5′-GCTCATCCCAATTGCAAAGT-3′	189	NM214390
	Antisense	5′-TTCCTCCAGCTCCTCACATT-3′		
MCP1	Sense	5′-TCTCCAGTCACCTGCTGCTA-3′	210	X79416
	Antisense	5′-AGGCTTCGGAGTTTGGTTTT-3′		
VEGF	Sense	5′-ATGGCAGAAGGAGACCAGAA-3′	224	AF318502
	Antisense	5′-ATGGCGATGTTGAACTCCTC-3′		
Haptoglobin	Sense	5′-AGAACCCAGTGGATCAGGTG-3′	234	NM214000
	Antisense	5′-CCTCCTGTTTCTTTCCCACA-3′		
MIF	Sense	5′-CGATGTTCGTGGTAAACACC-3′	235	DQ989235
	Antisense	5′-AGCAGCTTGCTGTAGGAACG-3′		
Lipoprotein Lipase	Sense	5′-TGGACGGTGACAGGAATGTA-3′	237	AF102859
	Antisense	5′-AAGGCTGTATCCCAGGAGGT-3′		
Cyclophilin	Sense	5′-ATGGTAACCCCACCGTCTTC-3′	376	AY008846
	Antisense	5′-GTTTGCCATCCAACCACTCAG-3′		

2.1. Real-time PCR analysis of gene expression

The adipokine genes selected for analysis included leptin, adiponectin, TNFα, IL-1β, IL-6, IL-8, IL-10, IL-15, haptoglobin, VEGF, MIF and MCP1. These genes were selected as they all have been demonstrated to be expressed by adipose tissue from mature animals or humans as described in the Introduction. Leptin, adiponectin and LPL were selected because they are markers for differentiation (McNeel et al., 2000). The interleukins-1β, 6, 8 and 15 were included because they have been identified by Hausman et al. (2006) to be expressed by neonatal pig adipose tissue using proteomics. Interleukin-10 expression was examined as IL-10 has been demonstrated to be expressed by porcine adipose tissue (Brix-Christensen et al., 2005), but more importantly it is considered to be an anti-inflammatory cytokine. Tumor necrosis factor α was examined as it has been the most characterized of all adipokines and has key regulatory roles in the expression of a variety of proteins. Several of the proteins that are regulated by TNFα include other adipokines, specifically, MIF, VEGF, MCP-1 and haptoglobin which were included in this study. Also, Hausman et al. (2006) have previously demonstrated the secretion of VEGF and TNFα by neonatal adipose tissue *in vitro*. Cyclophilin was used as a relative standard for comparisons. Preliminary experiments confirmed the expression of all of these genes by isolated adipocytes and also isolated stromal–vascular cells prepared from the adipose tissue of 21 day old pigs (data not presented).

The primers used for generating the adipokine amplicons are reported in Table 1. All primer sets were designed to span an intron. The amplicons were excised from an agarose gel, re-amplified, and run through a GenElute PCR clean-up kit (Sigma-Aldrich). The amplicons were subsequently sequenced to confirm identity using automated fluorescent DNA sequencing (ABI 310, Perkin Elmer Applied Biosystems, Foster City, CA, USA).

Thermal cycling and data acquisition were performed with a Bio-Rad iCycler IQ system (Bio-Rad Laboratories Inc., Hercules, CA, USA). Reverse transcription (RT) and real time PCR analysis were performed in a two tube assay. Reverse transcription (RT) was done using a Superscript First-Strand Synthesis System for RT-PCR kit (Invitrogen). Master mix was made containing random hexamers (50 ng/μL), 10 mM dNTP mix, RNase-free H_2O, and RNA (1 μg/μL). The RNA mix was annealed at 65 °C for 5 min. A second master mix was prepared with 10× RT buffer, 25 mM $MgCl_2$, 0.1 mM dithiothreitol, and 1.0 μL RNaseOut. This second master mix was added to the RNA mix and incubated at 25 °C for 2 min. Superscript II was then added and incubated at 25 °C for 10 min, 42 °C for 50 min, and 70 °C for 15 min. An aliquot of RNase H (1.0 μL) was then added and incubated at 37 °C for 20 min.

Real time PCR was done using the IQ sybr green supermix kit (Bio-Rad). A 24 μL reaction mix was made containing 12.5 μL sybr green supermix, 1.0 μL forward primer (10 μM), 1.0 μL reverse primer (10 μM) and 9.5 μL sterile water. This reaction mix was added to each well, followed by 1.0 μL RT product (25 μL total volume).

Parameters for all reactions except cyclophilin were as follows: 1 cycle 95 °C for 15 min (PCR activation), followed by 30 cycles, 94 °C for 15 s, 58 °C for 30 s, 72 °C for 30 s, with a final extension at 72 °C for 8 min. Parameters for cyclophilin were as follows: 1 cycle 95 °C for 15 min (PCR activation), followed by 30 cycles, 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, with a final extension at 72 °C for 7 min. Melting curve analysis was performed on all real time PCR reactions to confirm specificity and identity of the real time PCR products. A nontemplate control was run for every assay. Specificity of real time PCR products was further confirmed by agarose gel electrophoresis. The two-step real time PCR reactions were optimized for linearity (exponential amplification) from >20 to <30 cycles under the conditions described above.

2.2. Quantification of gene expression

At the end of the PCR, baseline and threshold crossing values (C_T) for all analyzed genes were calculated using the BioRad software and the C_T values were exported to Microsoft Excel for analysis. The relative expression of the genes of interest, standardized against the amount of cyclophilin mRNA, was calculated using the ΔΔCT method

(Winer et al., 1999; Livak and Schmittgen, 2001). Values are presented as the mean±SEM of duplicate determinations from tissues from six individual animals at each age.

2.3. Statistical analysis

Data were analyzed by one-way analysis of variance using SigmaStat software (SPSS Science, Chicago, IL, USA) to test for age effects within each tissue. Mean separation was analyzed using Student–Newman–Keuls test. Means were defined as significantly different at $P<0.05$.

3. Results

Leptin mRNA abundance within the SQ adipose tissue gradually increased with age from d1 to d21 (Fig. 1a). Perirenal adipose tissue could not be visually identified in d1 pigs with any certainty, so it could not be collected for this study. Leptin expression in PR adipose tissue gradually increased between d4 and d21. Similar to leptin, adiponectin mRNA abundance gradually increased with age from d1 to 21 in SQ, while adiponectin expression was only elevated at d21 in PR adipose tissue (Fig. 1b). These results paralleled the increase in LPL mRNA abundance observed in both sites of adipose tissue deposition (Fig. 1c).

The interleukins all displayed different developmental patterns of expression (Fig. 2). Interleukin-1β (Fig. 2a) mRNA abundance was lowest at d7 in the SQ adipose tissue but otherwise levels were unchanged through the study; while IL-1β mRNA abundance in PR adipose tissue gradually increased with age. Interleukin-6 expression was low and did not change in the SQ adipose tissue throughout the time of the study while the PR adipose tissue expression was elevated at d21 (Fig. 2b). The mRNA abundance for IL-8 was highest at d1 and rapidly dropped by d4 of age in the SQ adipose tissue (Fig. 2c). Interleukin-8 mRNA abundance in the PR at d4 was extremely low but increased 14-fold by d7 ($P<0.01$). Interleukin-10 (Fig. 2d) gene expression in the SQ adipose tissue declined at d7 but otherwise levels were relatively unchanged throughout the first 21 days of life. No apparent changes were detected in IL-10 mRNA abundance within PR adipose tissue with age. Interleukin-15 showed a gradual increase in mRNA abundance with age in both sites of adipose deposition (Fig. 2e), which paralleled the expression of LPL and leptin (Fig. 1).

Expression of TNFα and TNFα responsive genes did not present a consistent pattern of development (Fig. 3). For example, TNFα mRNA abundance did not change until d21 of age in either SQ or PR adipose tissue (Fig. 3a). In contrast, MCP1 mRNA abundance gradually increased in the PR adipose tissue, while expression in the SQ adipose tissue increased between d1 and d4 and no further through d21 (Fig. 3b). Meanwhile, haptoglobin (Fig. 3c) and VEGF (Fig. 3d) displayed reductions in SQ gene expression between d1 and d4 with no further change in expression; while age had no effect on the mRNA abundance for either gene in PR adipose tissue. The mRNA abundance of MIF decreased in both SQ and PR adipose tissue with age throughout the study (Fig. 3e).

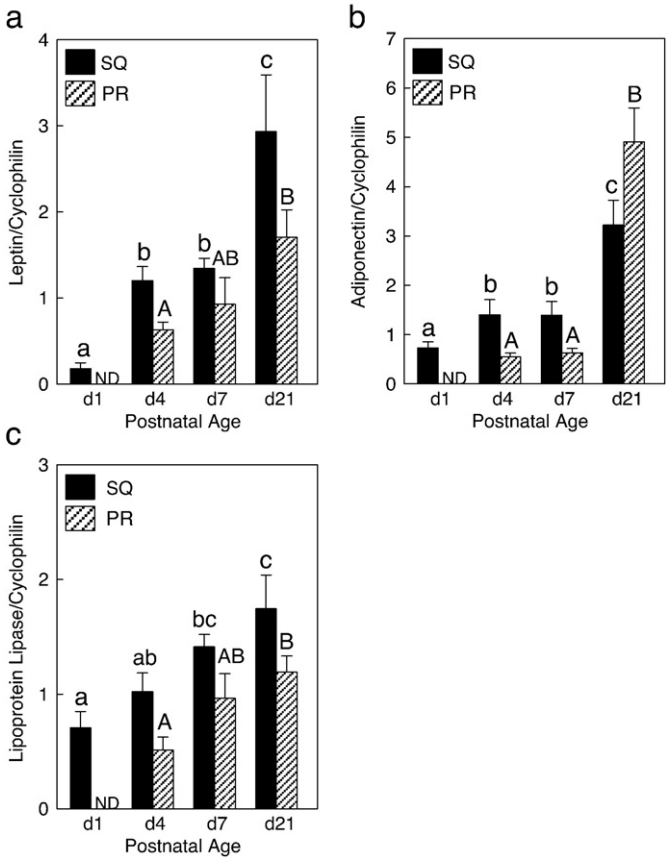

Fig. 1. Relative leptin (a), adiponectin (b) and lipoprotein lipase (c) expression in subcutaneous (SQ) and perirenal (PR) adipose tissues from neonatal pigs. Adipose tissue was collected from pigs at d1, d4, d7 or d21 of age. Reverse transcription and real time PCR were then performed as described in the methodology. Data are expressed relative to cyclophilin expression in each tissue sample. ND = not determined; ^{abcd} Columns not sharing a common superscript letter are different ($P<0.05$; $n=6$); ^{ABCD} Columns not sharing a common superscript letter are different ($P<0.05$; $n=6$).

4. Discussion

Numerous studies have described the expression of adipokines in adult animals and humans (Trayhurn and Wood, 2004; Trayhurn et al., 2006). Hausman et al. (2006) have previous reported that 5–7 day old pig adipose tissue expresses a variety of adipokines. However, the present study is the first attempt to describe the development of endogenous adipokine expression within neonatal adipose tissue *in vivo* for any species. The neonatal period is characterized by the rapid accumulation of lipid within newly formed adipocytes and the differentiation of preadipocytes into adipocytes (Martin et al., 1984; Herrera and Amusquivar, 2000). This is reflected in the accumulation of mRNA for LPL in the present study, which is a marker for adipocyte differentiation and lipid metabolism (Ailhaud, 1996; Ding et al., 1999; McNeel et al., 2000). Parallel to these increases in LPL mRNA abundance, leptin and adiponectin increased with age during the neonatal period. Both genes are expressed almost exclusively within the adipocyte in swine (Chen et al., 1997; Lord et al., 2005). Thus the increases in leptin, adiponectin and leptin during the first 21 days of life may reflect the formation of adipocytes and their rapid accumulation of lipid.

Ramsay and Richards (2005) have previously reported that leptin mRNA abundance is greater in SQ than PR adipose tissue of post-weaning swine; this appears to be true even during the neonatal period. In contrast, Lord et al. (2005) reported that intra-abdominal adipose tissue expresses higher levels of adiponectin than SQ adipose tissue in post-weaning swine. The present study suggests that this change in relative expression between sites of adipose tissue deposition does not happen until after d21 of age.

Cytokines are important components of overall adipokine production. The role of adipose tissue in the production and secretion of cytokines has been demonstrated over the past 20 years (Tilg and Moschen, 2006). The list of potential cytokines expressed by adipose tissue is quite large and only a fraction of the potential cytokine profile was examined in the present study, a selection based primarily on previous reports of expression by pig adipose tissue (Ajuwon et al., 2004; Hausman et al., 2006, 2007). Hausman et al. (2006) used microarray and proteomic analyses to demonstrate that neonatal (d5–7) pig adipose tissue expresses a large variety of cytokines.

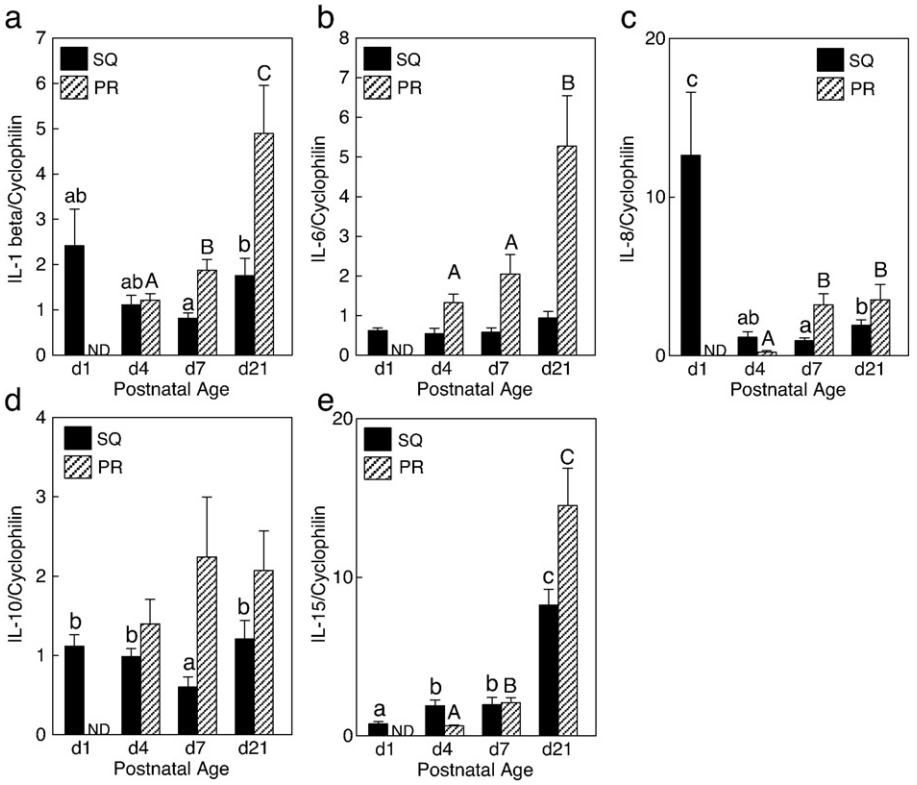

Fig. 2. Relative interleukin-1β (IL-1β, a), IL-10 (b), IL-6 (c) and IL-8 (IL-8; d) and interleukin-15 (IL-15, e) expression in subcutaneous (SQ) and perirenal (PR) adipose tissues from neonatal pigs. Adipose tissue was collected from pigs at d1, d4, d7 or d21 of age. Reverse transcription and real time PCR were then performed as described in the methodology. Data are expressed relative to cyclophilin expression in each tissue sample. ND = not determined; [abcd] Columns not sharing a common superscript letter are different ($P<0.05$; $n=6$); [ABCD] Columns not sharing a common superscript letter are different ($P<0.05$; $n=6$).

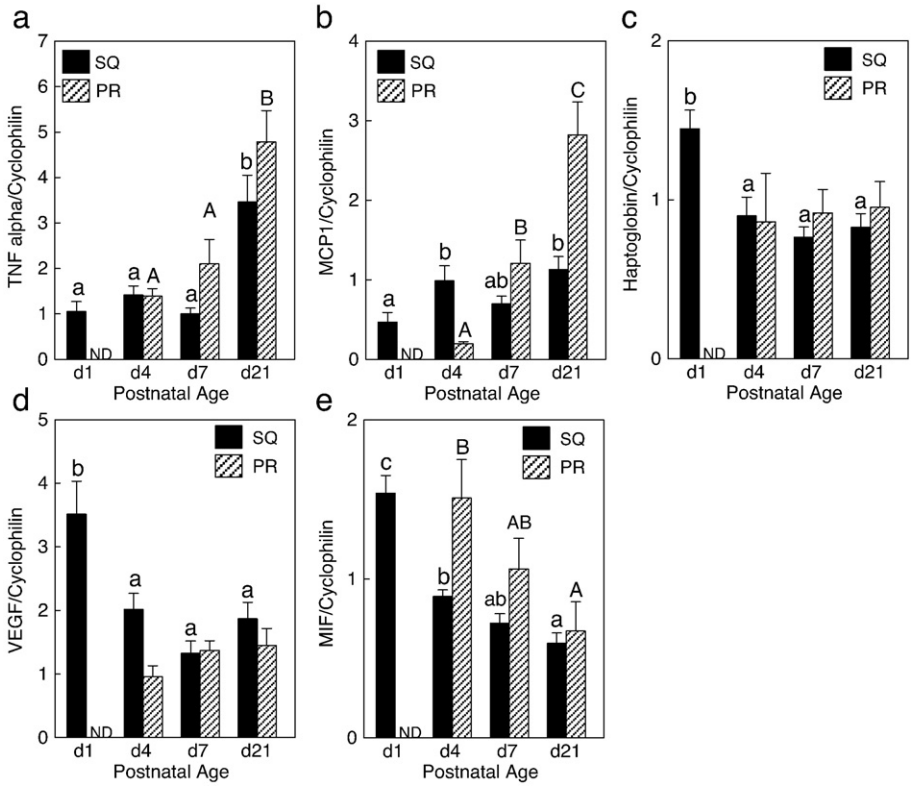

Fig. 3. Relative tumor necrosis factor α (TNFα, a), monocyte chemoattractant protein-1 (MCP1; b), haptoglobin (c), vascular endothelial growth factor (VEGF, d) and macrophage migration inhibitory factor (MIF; e) expression in subcutaneous (SQ) and perirenal (PR) adipose tissues from neonatal pigs. Adipose tissue was collected from pigs at d1, d4, d7 or d21 of age. Reverse transcription and real time PCR were then performed as described in the methodology. Data are expressed relative to cyclophilin expression in each tissue sample. ND = not determined; [abcd] Columns not sharing a common superscript letter are different ($P<0.05$; $n=6$); [ABCD] Columns not sharing a common superscript letter are different ($P<0.05$; $n=6$).

Ajuwon et al. (2004) have previously reported that adipocytes can secrete IL-6 when stimulated with lipopolysaccharide (LPS). The present study only looked under basal conditions and not when the cells were stimulated or challenged by any mechanism. Interleukin 6 has been demonstrated to be induced by fatty acid (Ajuwon and Spurlock, 2005) and by TNFα (Wang and Trayhurn, 2006). Therefore, one would predict that the IL-6 expression would be high in the neonatal pig and increase during lactation. This was not observed in the intact SQ, but these factors could contribute to the changes in IL-6 in the PR of the neonatal pig; IL-6 can stimulate glucose transport in 3T3-L1 adipocytes (Stouthard et al., 1996), which provides energy for lipid accretion and could contribute to the increase in lipid content in the PR.

Despite the effects of IL-6 on glucose metabolism, the primary function of these interleukins is in the immune stress response which can impact adipose tissue due to the variety of immune responsive cells (macrophages, mast cells, monocytes and endothelial cells) present within the tissue Fain (2006); this immune response can then impact adipose tissue metabolism (Trayhurn and Wood, 2004; Tilg and Moschen, 2006; Trayhurn et al., 2006). In any case, the interleukins all responded differently with the development of neonatal porcine adipose tissue. This would imply that they are regulated by distinct mechanisms. In addition as exemplified by IL-6 above, the interleukin data suggest that the two sites of adipose tissue deposition are subject to different mechanisms of regulation. The limited developmental changes in IL-1β and IL-6 mRNA abundance within the SQ adipose tissue relative to the significant changes detected in PR adipose tissue provide clear evidence for this. Interestingly, IL-1β has been demonstrated to induce IL-6 in adipocytes (Flower et al., 2003) and may contribute to the observed pattern of expression for IL-6. Numerous studies have demonstrated that intra-abdominal adipose tissue and subcutaneous adipose tissue differ metabolically and respond differently to endocrine stimuli in post-weaning animals and humans (Anderson et al., 1972; Wajchenberg et al., 2002; Lafontan and Berlan, 2003; Rattarasarn, 2006). This is now also apparent at the very earliest stages of postnatal development in the adipokines expressed by the neonatal pig.

Interleukin-10 was the only anti-inflammatory adipokine examined in this study. Interleukin-10 has been reported to down-regulate TNFα, IL-6 and IL-8 in neonatal whole blood cultures (Schultz et al., 2004), although to a lesser extent than in adult whole blood cultures. In addition, Schultz et al. (2004) reported that leukocytes from neonates have a diminished IL-10 response to LPS than leukocytes from adults. The data from the present study is in agreement with Schultz et al. (2004) as the mRNA abundance for IL-10 did not dramatically change in either SQ or PR adipose tissues during the first 21 postnatal days of age. Dietary fatty acids have been reported to inhibit IL-10 expression (Bradley et al., 2008). Thus, the high fat diet of the pre-weaned pig may contribute to the absence of any change in IL-10 mRNA abundance during the first 21 days postnatal.

Despite the lack of change in the anti-inflammatory IL-10 mRNA abundance, the expression of the pro-inflammatory adipokines TNFα and IL-15 increased in both SQ and PR adipose tissues with age. The mRNA abundance of IL-1β, IL-6, and to a less extent IL-8, also increased with age in PR adipose tissue. As IL-10 is an anti-inflammatory cytokine, these data would suggest that the observed increase in inflammatory cytokine gene expression with the absence of a counter regulatory IL-10 response may produce a net increase in inflammatory status in the young pig.

Interleukin-15 expression has been previously shown in porcine adipocytes *in vitro* (Ajuwon et al., 2004). In the present study, IL-15 mRNA abundance paralleled the expression of leptin and LPL, suggesting that IL-15 expression is associated with the differentiation or the metabolism of adipocytes. Previous research has indicated that IL-15 is lipolytic to porcine adipocytes (Ajuwon and Spurlock, 2004). The parallel increases in IL-15, leptin and LPL reported here may indicate that IL-15 expression is associated with the metabolic development of the adipocyte.

Tumor necrosis factor α has been the best characterized adipokine for its metabolic actions on adipose tissue. For example, TNFα can promote lipolysis, suppress lipogenesis and inhibit lipid uptake by adipose tissue (Price et al., 1986; Ruan and Lodish, 2003). If TNFα were highly expressed within neonatal pig adipose tissue, it could inhibit the accumulation of adipose tissue and consequently have deleterious effects on neonatal survival. In support of this hypothesis, TNFα supplementation to primary cultures of neonatal pig adipose tissue reduced the differentiation of preadipocytes, thus reducing the potential number of adipocytes (Boone et al., 2000). However, the present data demonstrate that TNFα mRNA abundance remains relatively low through the first critical week of life, but then increases after energy storage in adipose tissue has developed (Anderson and Kauffman, 1973; Mersmann et al., 1975). Fain et al. (2004a,b) have reported that TNFα secretion is greater from intra-abdominal adipose than SQ adipose tissue. Tumor necrosis α mRNA abundance in the intra-abdominal PR adipose tissue was greater than in the SQ adipose tissue of neonatal pigs in the present study and therefore in agreement with Fain et al. (2004a,b).

As previously mentioned, TNFα can regulate the expression of a number of adipokines, including MIF (Atsumi et al., 2007; Hirokawa et al., 1997), MCP1, haptoglobin and VEGF (Wang and Trayhurn, 2006). This would lead to the prediction that expression of these genes would change as TNFα expression increased between d7 and d21. However this did not occur with haptoglobin, VEGF or MIF mRNA abundance in the SQ or PR. However, MCP1 expression in PR does parallel the changes in TNFα expression. MCP1 has been demonstrated to reduce insulin stimulated glucose transport in 3T3-L1 adipocytes (Sartipy and Loskutoff, 2003), and thus may function along with TNFα in inhibiting the supply of energy for lipid synthesis. Taking all of these data into account, TNFα regulation of MIF, haptoglobin and VEGF does not appear to be functional at this early age in pig adipose tissue development or TNFα is not a primary regulator of the expression of these adipokines in the neonate.

A possible explanation for the change in relative gene expression for haptoglobin, MIF and VEGF between d1 and d4 may be related to the metabolic changes that the neonate experiences in the transition from the carbohydrate based nutrition of the fetus to the fat based nutrition of the suckling pig. The adipose tissue is the primary energy storage organ during this transition. In addition, this early neonatal period is accompanied by major changes in the endocrinology of the pig, which may impact gene expression in the adipose tissue. For example, large reductions in serum cortisol and growth hormone occur within the first few days of birth in pigs, while increases in serum insulin and thyroid hormones have been reported (Herbein et al., 1977; Scanes et al., 1987; Llamas Moya et al., 2007). However, the inverse response by genes specific to adipocytes (leptin, adiponectin) raises the question as to whether the reductions in VEGF, haptoglobin and MIF mRNA abundance are the consequence of interaction with these systemic hormones, or are more related to paracrine or autocrine regulation by factors within the adipose tissue.

While the function of haptoglobin within adipose tissue is unknown, VEGF functions as a promoter of vascularization, an essential process in adipose tissue development (Hausman, 1985). Following the reduction in expression between d1 and d4, VEGF mRNA abundance does not really change, indicating that other promoters of vascularization (Thalmann and Meier, 2007) may play important roles in neonatal adipose tissue development. A role for MIF in adipocyte metabolism has been clearly established with its primary function appearing to be inhibiting insulin signaling and glucose transport (Atsumi et al., 2007); therefore a reduction in the expression of this gene would lead to an augmentation of lipid accretion within neonatal adipose tissue.

This study is the first to characterize the initial development of adipokines following birth. Previous work by Hausman et al. (2006) demonstrated expression of many cytokine proteins in adipose tissue

from the d5–7 pig, but a developmental pattern could not be ascertained; while Llamas Moya et al. (2007) recently described the ontogeny of plasma TNFα, IL-1β and haptoglobin in the pig. The present study specifically characterized the development of adipokine expression by the SQ and PR adipose tissues from d1 to d21 of age. This study only examined mRNA abundance and did not demonstrate any protein production. However, Hausman et al. (2006) reported the expression of IL-1β, IL-6, IL-8, IL-15, TNFα, VEGF adiponectin and other adipokines by neonatal stromal–vascular cultures, but it is unknown whether the other gene products examined in the present study are translated and the proteins secreted by porcine adipose tissue. Nevertheless, all of these proteins have been demonstrated to be secreted by adipose tissue from other species (Fain, 2006). The contribution of these adipokines to overall serum levels of these proteins is questionable (Fain, 2006), but their potential role in adipose function is undeniable. Further research is necessary to determine if these adipokine genes respond to stressors (environmental, immunological or nutritional) and what impact these adipokines might have on neonatal adipose function and development.

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